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Evidence exists that women exercising have lower estrogen levels than sedentary women. These lower estrogen levels may be the mechanism behind their reduced breast cancer risk. Previous studies included athletes with high exercise levels, and estrogen measurements were based on a few serum samples from different times during a menstrual cycle. This study includes identical female twins who are discordant for moderate exercise. Estradiol is measured on a daily basis from saliva samples collected during a complete menstrual cycle. Procedures and questionnaires have been developed; enrollment of eligible pairs is ongoing. Screening interviews have been conducted with 197 pairs. Of these, 36 were initially eligible; however 7 declined to participate and 2 later became ineligible due to menopausal related reasons. Thus, sample collection is currently completed or underway for 27 pairs. Estradiol and progesterone assays have been completed for 16 pairs. Although fewer eligible pairs were identified than expected, more twins will be available from additional phases of the California Twin Program. In Year 3, we will continue to screen and enroll twins, complete data entry of questionnaires, conduct hormonal assays, integrate laboratory and questionnaire data sets, and complete preliminary analyses. It is anticipated that a 1 year no cost extension will be requested.

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FOREWORD

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Ann A Hamilton 10/4/1999

PI - Signature Date

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5) INTRODUCTION

The purposes of the study are the following:

(1) To determine the effect of moderate exercise on E2 levels during the follicular and luteal phases of (ovular) menstrual cycles by means of daily salivary samples in healthy premenopausal identical twins who differ in their amount of physical exercise activity per week.

(2) To determine the effect of moderate exercise on frequency of anovulation and on menstrual cycle length (specifically luteal phase length) in identical twins who differ in their amount of physical exercise activity per week.

Overview: Exercise has been shown to be associated with a reduced risk of breast cancer [4,5,8,10,11]. There is evidence that women exercising, for an hour or more per day, have lower serum estrogen (estradiol) levels than sedentary women (due to more anovular cycles and lower estrogen levels in ovular cycles). These lower estrogen levels appear most likely to be the mechanism behind their reduced breast cancer risk, however much is still unknown. Previous studies have, for the most part, focused on the effects of high exercise levels among athletes, as opposed to more moderate levels of exercise, on estrogen levels, and they may have been subject to 'selection bias', i.e. women who exercise may do so because of predisposing hormonal factors. In addition, the estradiol measurements have usually been based on only a few serum samples taken at different times during a menstrual cycle. This study is addressing these issues by using 60 sets of monozygous twins who are discordant with regard to moderate exercise habits (i.e. sedentary vs. exercising an average of 20 minutes/day), but are identical for heritable aspects of body build and constitution. Estradiol is being measured on a daily basis by use of salivary samples collected during a complete menstrual cycle. The subjects are being selected from pairs of healthy premenopausal identical twins under the age of 45 who participated in the California Twin Cohort Study. They are being screened to determine eligibility (i.e. neither twin having an endocrine or metabolic disorder and the pair discordant for current amount of physical exercise activity), before being asked to participate. The use of the salivary samples is an innovative method for the measurement of estradiol and offers distinct advantages over the more traditional serum hormone measurements for which daily samples are not practical. Repeated sampling, as compared to single or infrequent sampling of individuals makes it possible to more accurately characterize ovarian function and allows for a more complete assessment of estradiol levels over different phases of the menstrual cycle, without the discomfort of venipuncture or the inconvenience of office visits. Salivary steroids have been shown to be extremely stable when samples are properly treated and this method of collection is ideally suited for use in the proposed study where subjects are located throughout California[43-46]. The hormone assays are being done by Dr. Peter Ellison (Co-Investigator), an expert in the analysis of and validation of salivary samples. We are also obtaining information on daily physical exercise activity during the month of sample collection and dietary intake using established and well tested questionnaires. Analysis of covariance methods will be used to assess the relationship of estrogen levels during different parts

of the menstrual cycle to exercise, controlling for diet, body mass, and other potentially confounding factors. Based on the sample size of 60 pairs of twins, we have the power to detect differences in estradiol levels of 15% between the sedentary and moderately exercising twins. The study has important public health implications in developing strategies for the prevention of breast cancer.

6) BODY

Technical Objectives and Work Accomplished in year 2:

Technical objectives 1-4: Selection of twins and collection of saliva samples: Ongoing throughout Yrs. 1, 2, and during the first 6 months of Year 3.

1. During the course of the study identical female twins will be selected who previously participated in the California Twin Cohort and indicated that they are premenopausal.
2. These pairs will be called on the telephone and re-interviewed regarding factors related to their eligibility.
3. Once a pair is determined to be eligible and they agree to participate they will be mailed informed consent forms, saliva sample collection kits, and exercise and dietary questionnaires.
4. We will check with them periodically to determine when the first day of their period occurs and assure that they are following the directions for collection of the saliva samples.
5. They will mail their completed sample kits to Dr. Ellison's laboratory and the completed questionnaires to USC.

Work accomplished on these objectives:

We originally selected 182 identical exercise discordant female pairs from the California Twin Cohort who were born before 1957 and were part of the first group of twins sent questionnaires in 1991-1992. During 1998 questionnaires were sent to additional California Cohort twins born before 1965 and 39 pairs have been selected from this group where both members of the pair participated. Of these 221 pairs, screening interviews have been conducted with 197 (Table 1), leaving 24 pairs still in process. For 10 pairs of twins, one or both members declined the screening interview. Among the 195 are 32 pairs currently classified as lost; however we plan to implement more tracing methods (i.e. Experian) to attempt to locate them. From the 197 pairs, 36 (18.3%) were initially identified as eligible for the study; however 7 declined to participate (2 of these after receiving the kits) and 2 others later became ineligible due to menopausal related reasons (i.e. started taking HRT's). Thus there are currently 27 pairs (13.7%) who have either completed the saliva collection process or are in process (Table 1).

Of those determined to be ineligible, the most common reasons were use of OC's or hormones (38) followed by parity discordance (i.e. one twin parous, the other nulliparous) (24), and one or both twins menopausal (14).

Due to higher rates of ineligibility than anticipated we have had fewer pairs participate than expected. However an additional group of 30,000 younger twins (i.e. those born between 1965-1971) are being sent the California Twin Cohort questionnaire within the next few weeks (i.e. October, 1999). Thus, in addition to the 26 pairs we are still screening and the 32 currently 'lost' pairs, we expect to have another 40-50 pairs to contact from the new group. In order to achieve as large a sample size as possible, we anticipate making a request for a one year no cost extension. During that time additional twin pairs are expected to become available as more phases of the California Twin Cohort are implemented.

Table 1: Results of Screening Interviews

Result of Screening of Both Members of Pair	Number of Pairs	Percent of Total Pairs Screened
Eligible	36	18.3
And participating	(27)	(13.7)
And declined participation	(7)	(3.6)
And later became ineligible	(2)	(1.0)
Screened and not eligible because:	119	60.4
1+ had menopause	(14)	(7.1)
1+ had very irregular periods	(2)	(1.0)
Parity discordant	(24)	(12.2)
1 + had disqualifying disease	(6)	(3.0)
1+ taking OC's or hormones	(38)	(19.3)
1+ taking cortisone/prednisone	(7)	(3.6)
1+ breast fed a child or pregnant within past year	(8)	(4.0)
Multiple of above reasons	(10)	(5.1)
Both had same exercise level	(9)	(4.6)
One twin was deceased	(1)	(0.5)
Lost, could not screen	32	16.2
Refused screening interview	10	5.1
Total	197	100.0

Technical Objective 5: Completion of Hormonal Assays: Year 1, month 3 through Year 3, Month 9.

1. Dr.Ellison's Laboratory will receive the kits and will be blinded as to which twin is performing more exercise.
2. The laboratory assistant will complete the hormonal assays according to standard protocols.
3. Results will be sent to USC.

Dr. Ellison's Laboratory has processed samples from 16 pairs to date. Assays have been completed for daily estradiol levels (Attachment 1) and the mean midluteal progesterone levels. One twin was determined to have had a non-ovulatory cycle from these results. Two additional pairs completed the collection and sent the samples to Harvard; however their samples were not usable due to too many skipped days during the month. Another twin sent in saliva samples that contained only ¼-1/3 the volume requested. As this will not be sufficient for analysis we will ask her if she will try again. We plan to also as the twin with the anovulatory cycle to repeat the collection if possible.

If we complete the study with fewer completed pairs than expected, additional, more detailed daily analysis of progesterone levels may be done on the samples we have at the end of the project.

Technical Objectives 6-7: Data Management: Year 1, Month 6-Year 3 Month 10
1. Physical Activity questionnaires will be coded and entered at USC.
2. Dietary questionnaires will be sent to Dr. Willett for analysis, with results being sent to USC.
3. Hormonal assay data will be merged with the questionnaire data.

An Access data entry program has been created for the questionnaire data. The dietary questionnaires received so far are being sent to Dr. Willett, and plans for integrating the hormonal and physical activity questionnaire data are being finalized. The activities are being coded according to the 'Compendium of Physical Activities' [57] which is a coding system that classifies the energy cost of physical activities.

Technical Objectives 8-9: Data analysis and publishing of papers: Year 1, Month 12-Year 3, Month 12.
1. Preliminary and final analyses will be performed to address the stated hypotheses.
2. Papers will be published on the results.

Work has not yet begun on these objectives, but plans are in process.

7) Key Research Accomplishments

- We have demonstrated that the large majority of women participating are able to collect the saliva samples as requested and mail them to the Laboratory for analysis.
- We have developed the study materials and protocols

8) Reportable Outcomes

Since this is currently a work in progress we do not have reportable outcomes at this time. Results will be presented at the Era of Hope meeting in 2000. Due to the successful collaboration in this study with Dr. Ellison, a second grant involving use of saliva assays has been submitted (to the state of California) to study testosterone levels in males at risk of testis cancer. A student

interested in exercise physiology received training in research on this grant as she contacted the twins and conducted the screening interviews. At Dr. Ellison's Laboratory, a graduate student participated in the estradiol assays.

9) Conclusions

The first two years of the study have included development of study procedures, questionnaires, and coordination of work with Dr. Ellison's Laboratory. Screening interviews have been conducted with 197 pairs. In general, the eligible twins who agreed to participate (31 pairs so far) are willing to complete the rather demanding requirements of the study; however we have had 4 pairs drop out due to refusal (2) and development of an ineligible condition (2). Three of the 27 participating pairs have produced samples that are not acceptable and we will attempt to have the twins repeat the collection. One twin had an anovulatory cycle will be asked to repeat the cycle. Additional twins will soon be available for screening and participation due to the implementation of the third phase of the California Twin Program (funded by other sources). In the next year, we will continue to screen and enroll twins for participation, data entry of questionnaires will be completed, a batch of completed Willett dietary assessments will be sent for analysis, and we expect to have the laboratory hormonal assays completed on all pairs who have participated. Procedures will be developed for merging the data from these various sources into a unified record and preliminary analyses will be completed. We anticipate requesting a 1 year no cost extension to complete the sample collection.

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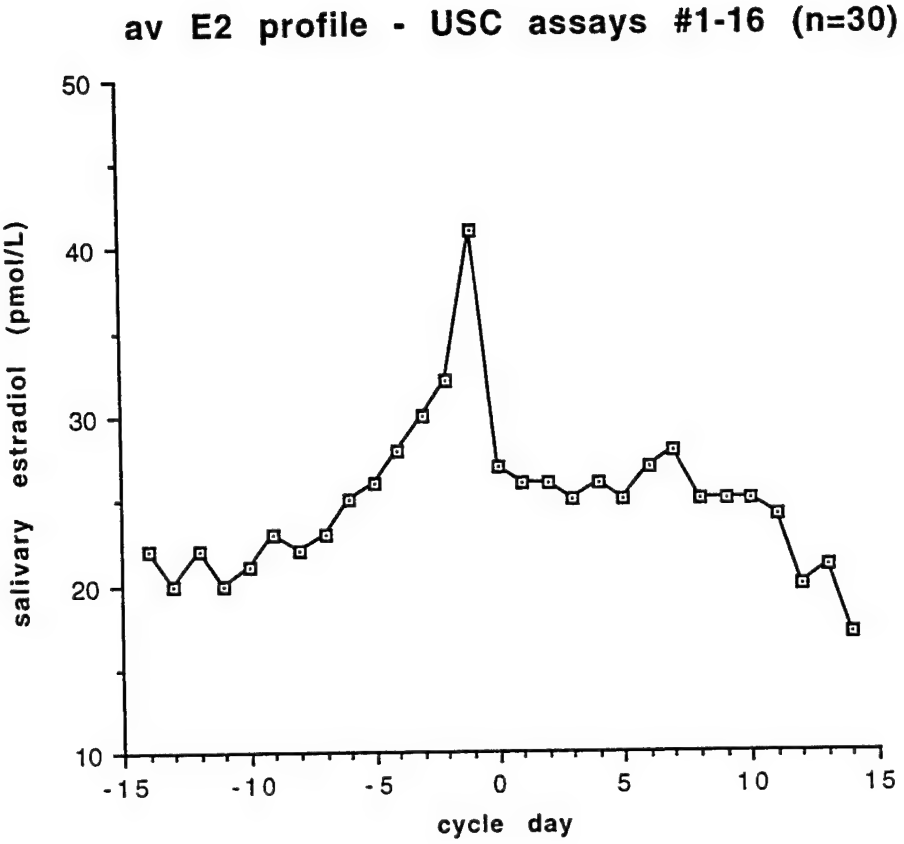
10) APPENDICES

1. Estradiol Assays for 16 pairs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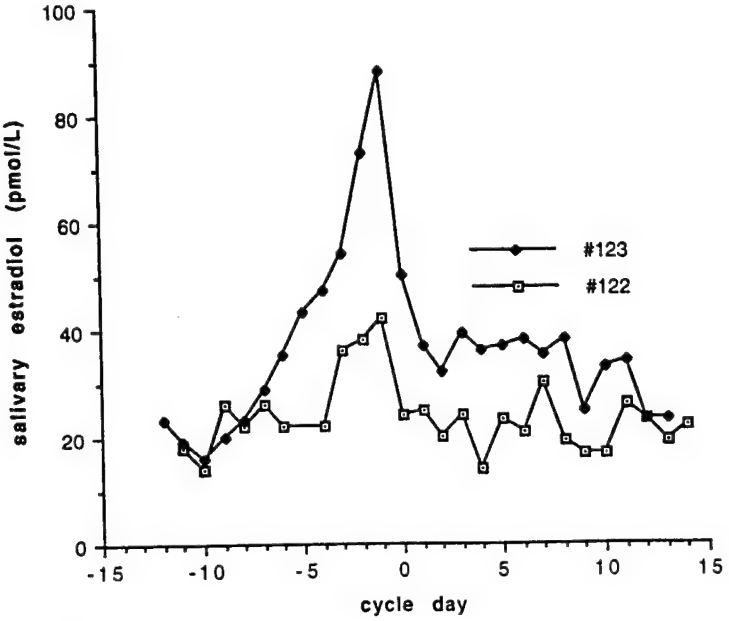
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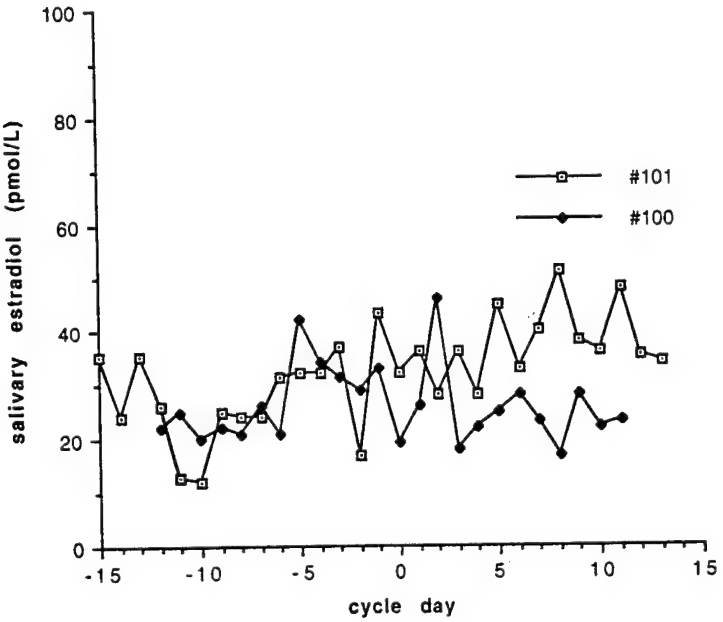
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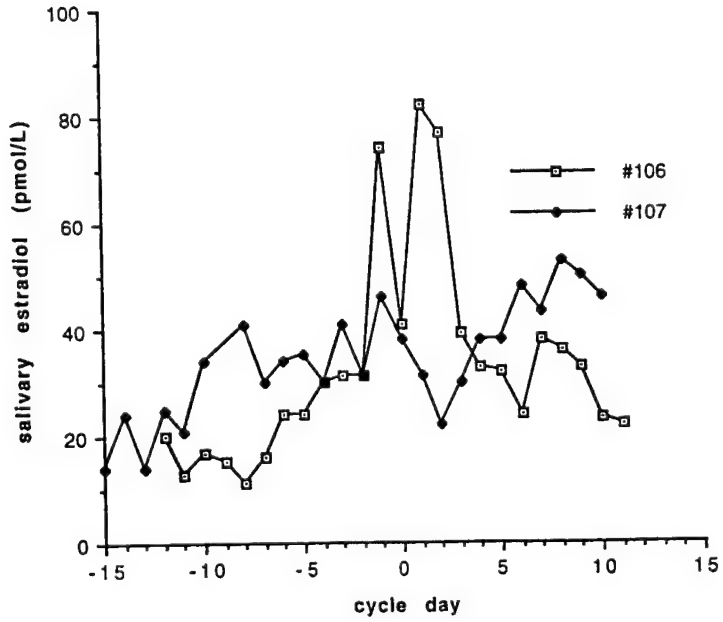
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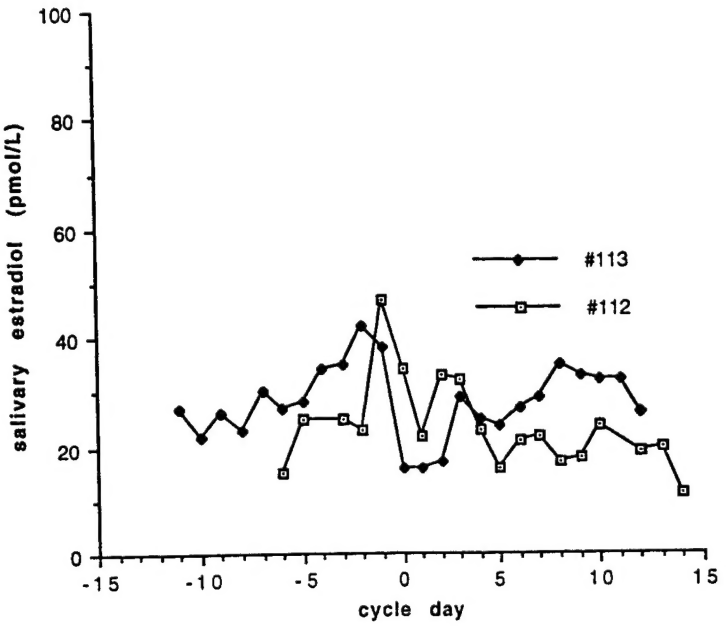
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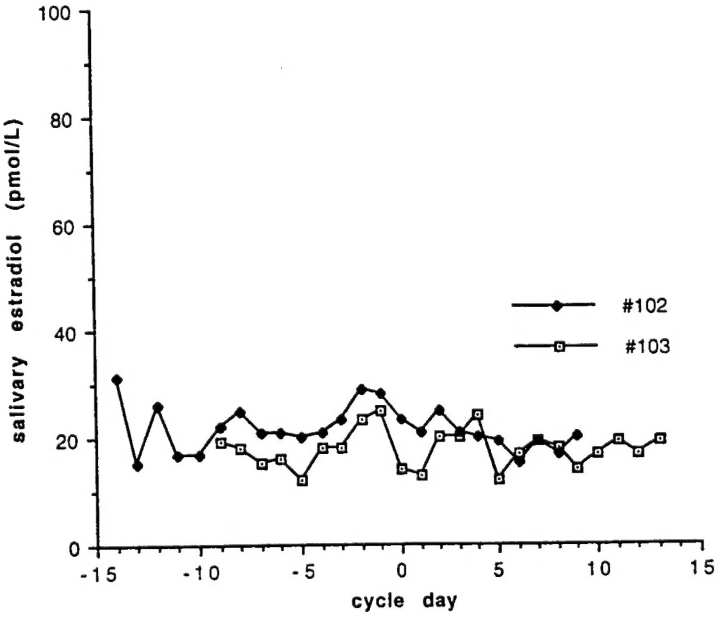
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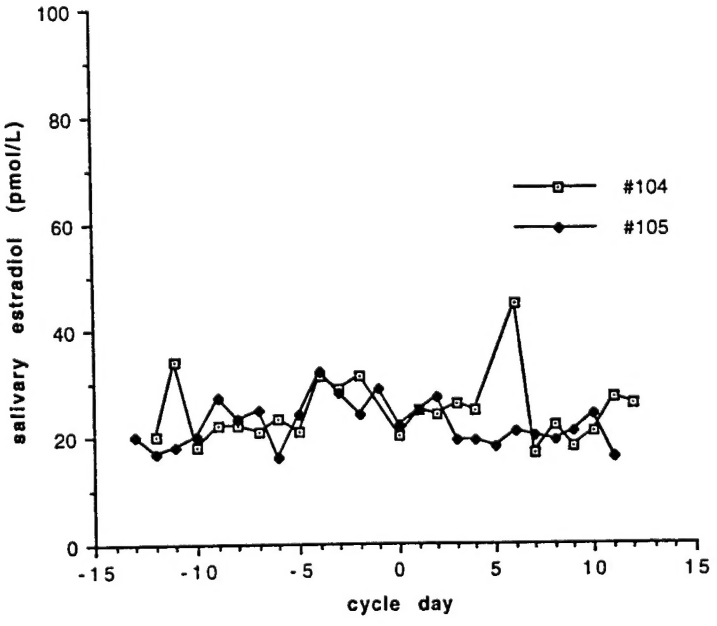
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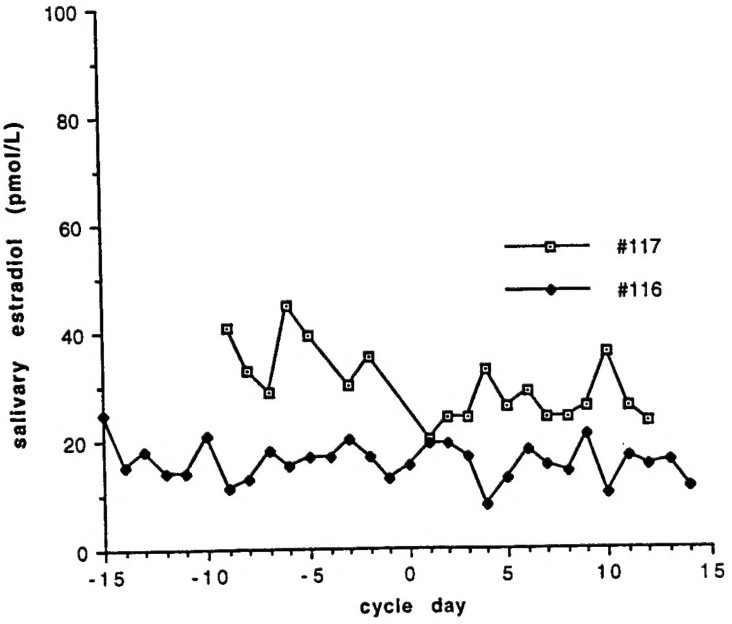
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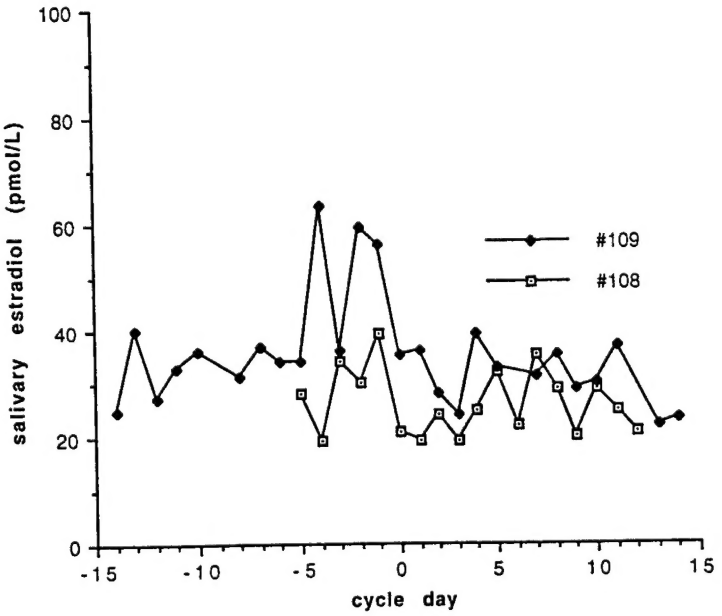
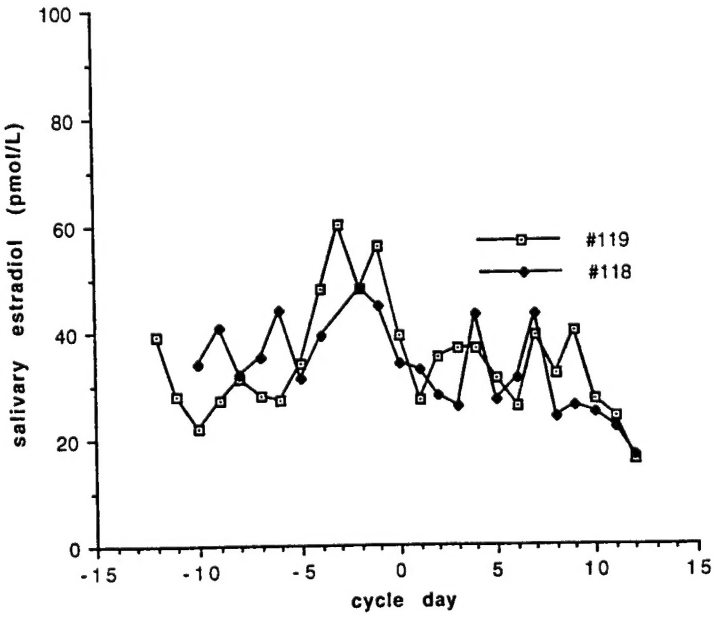
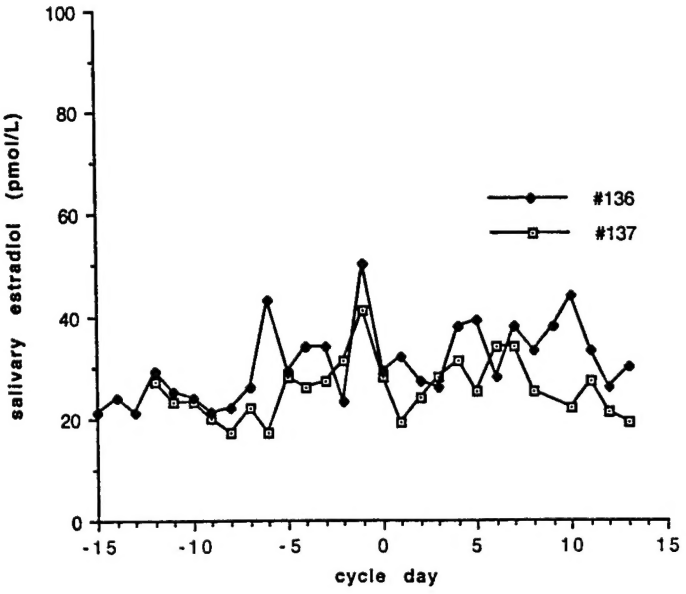
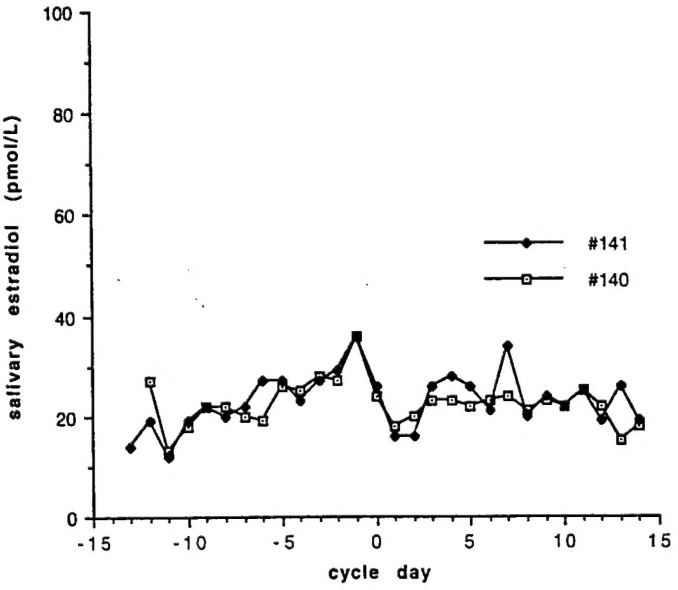
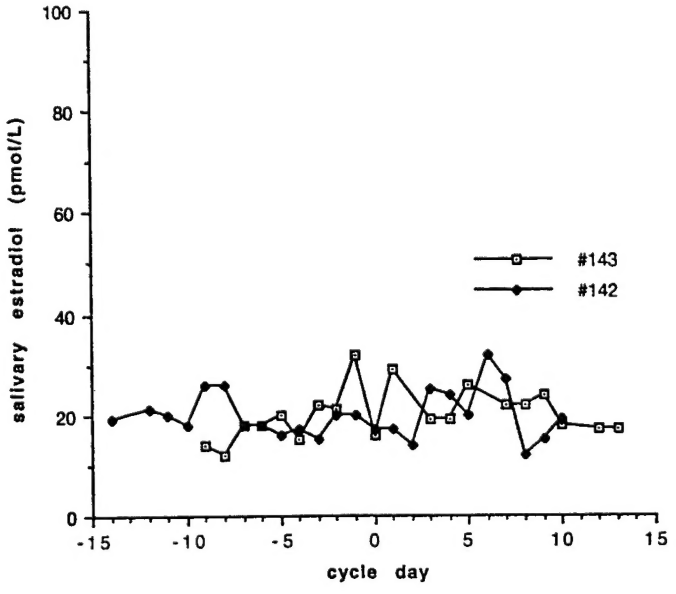
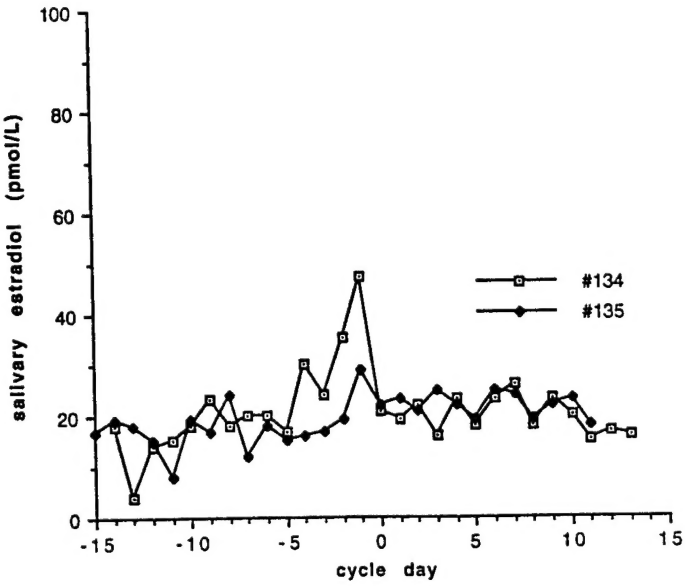

salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#109
#108

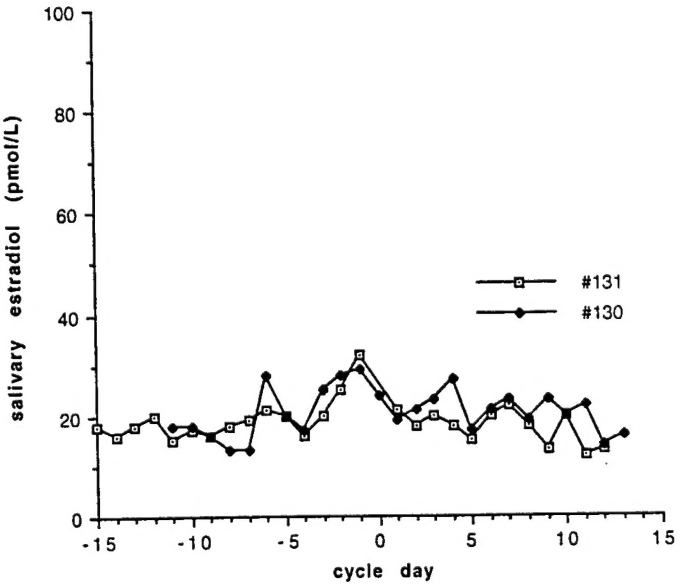

salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#119
#118

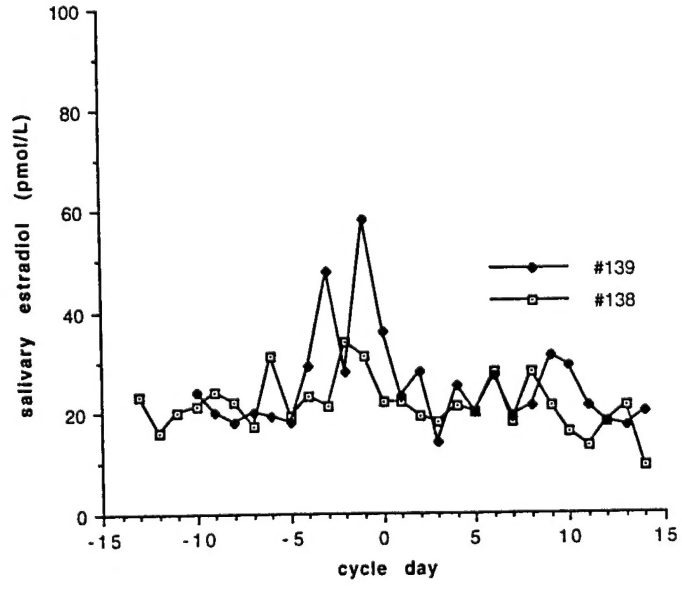

salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#136
#137


salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#141
#140


salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#143
#142


salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#134
#135


salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#131
#130


salivary estradiol (pmol/L)
100
80
60
40
20
0
-15
-10
-5
0
5
10
15
cycle day
#139
#138